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14. ABSTRACT
Positive surgical margins after radical prostatectomy can increase the risk of disease recurrence, and the preservation of erectile function depends on identification and careful preservation of the neurovascular bundles. Coherent anti-Stokes Raman scattering (CARS) is a powerful imaging modality with label-free and chemically selective imaging ability. Our group is pioneering the development of a miniaturized fiber-based CARS system to assist label-free differentiation of prostate tissues and cavernous nerves (CNs) during robotic-assisted radical prostatectomy. We have completed the design of a handheld CARS microendoscope probe which includes a customized micro-electromechanical systems (MEMS) scanning mirror as well as miniature optical and mechanical components. The microendoscope probe is currently in the phase of completing the fabrication. The transversal diameter of the probe is 14 mm which is the smallest among all reported CARS microendoscope probes. We have performed Monte Carlo simulation and are expecting 1 μm resolution for the CARS microendoscope probe system. To further improve the efficiency of laser delivery and signal collection for the fiber-based CARS system, we have tested the performance of fiber bundles in 6 and 18-multimode fiber configurations as well as single fiber. We have shown the feasibility of fiber-based systems by successful imaging of polystyrene beads and *ex vivo* tissue samples.

15. SUBJECT TERMS
 coherent anti-Stokes Raman scattering; endoscopy; endomicroscopy; fiber optics; micro-electromechanical systems

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Introduction

The study is based on label-free differentiation of prostate cancer tissues and cavernous nerves (CNs) by Coherent Anti-Stokes Raman scattering (CARS) technique. Label-free CARS imaging is promising for replacing biopsy and potentially offers new strategies for intraoperative and surgical applications. However, current CARS systems are built with bulky free-space optics and are not feasible for clinical use as the minimal size is a key requirement for development of a medical device for prostatectomy surgery. We have proposed to develop a miniaturized device by refining the original bulky CARS system into a compact all fiber CARS microendoscope to visualize prostate and cancer tissues during prostatectomy. We replaced the original bulky free-space optics with their fiber optic counterparts. The specific aims have not been significantly changed from the original approved statement of work.

Body

Aim 1: To refine our existing CARS microendoscope into a fiber device for *in vivo* cancer imaging.

A. Refine laser, detection, and data acquisition (DAQ) subsystems with fiber optic components.

We are planning to refine our original bulky free-space optical CARS system into a compact all fiber CARS microendoscope. The system will consist of a laser system, a detection system, and a fiber probe. In the past calendar year, we have tested collection efficiency and CARS imaging for fiber-based imaging systems. The work has led to two conference proceedings papers [1-2]. We are currently contacting TOPTICA Photonics Inc. to purchase fiber lasers. Aim 1A will be finished after we receive the fiber lasers and adjust the optical path of the CARS time delay line.

We tested the CARS single fiber (SMF28) signal collection efficiency with the same polarization control scheme to suppress four-wave-mixing noise as presented previously [3]. The CARS images of 10 µm polystyrene beads (PEBs) collected by the single fiber are shown in Figure 1 (Row C) and the intensity profile along the green line in each image is shown in Figure 2. We also tested the collection efficiency of the original bulky 60X objective at the same condition but before coupling to the single fiber for comparison (shown in Figure 1 Row B). By integrating the intensity of each image, we obtained the single fiber collection efficiency compared to the 60x objective. The single fiber could collect 0.73% of the signal collected by the 60x objective. To illustrate the tissue imaging ability of the single fiber, we utilized the single fiber system to image mouse adipocyte tissue and the CARS image collected by the single fiber is shown in Figure 3. The edges of the cells are revealed by single fiber collection, indicating our single fiber system as a feasible means for miniaturized optical imaging.

As an alternative, we examined the collection efficiency of a customized fiber bundle composed of 6 multimode fibers (MMFs) with the same polarization control scheme (Figure 1 Row B). The intensity profile along the green line in fiber bundle imaging result is also shown in Figure 3. By integrating the intensity of each image, we have shown that 6 MMF can collect 10.40% of that collected by the 60x objective, which is more efficient compared to 0.73% by the single fiber. Sample CARS images of mouse adipocyte and mouse lung tissue collected by the 6 MMF bundle are shown in Figure 4. To further optimize the collection efficiency of the fiber bundle, we increased the number of collection fibers to 18 and improved the collection efficiency to 15.22%. The imaging result of mouse adipocyte tissue by the 18 MMFs is shown in Figure 5. Our results indicate that fiber bundles have better collection efficiency and imaging quality than a single fiber system, which leads to the proposal we are currently writing: imaging of cancer stroma and tumor microenvironment by fiber bundle based label-free and chemical-selective microendoscope.

B. Develop a fiber probe based on microelectromechanical systems (MEMS) technology and consisting of a polarization maintaining fiber, a MEMS scanning mirror, and micro-optics.

We have completed Aim 1B in the past calendar year. We have finished the design of a handheld CARS microendoscope probe which uses a customized MEMS scanning mirror as well as miniature optical and mechanical components. The miniature probe is currently under fabrication and is epected to be available in the Spring of 2014 according to the fabrication schedule. We have been accepted to present the design principle of our miniaturized probe at the 2014 conference of Society of Photo-Optical Instrumentation Engineers (SPIE) Photonics West [4].

The optical imaging path of the miniature fiber optic probe is illustrated in Figure 6. Robot-assisted radical prostatectomies are now the most common surgical procedure for prostate cancer in the United States. Therefore, we designed the microendoscope probe to be less than 3 mm in diameter at the distal end. The CARS excitation laser emerging from the fiber is coupled into the collimator system with suitable numerical aperture match. The collimated light is scanned by the MEMS mirror and then is projected to the micro-objective subsystem which is aimed to achieve a large NA (numerical aperture) and high light coupling efficiency . The micro-objective subsystem contains an achromatic wide angle keplerian telescope beam expander and light focusing subsystem. The achromatic wide angle keplerian telescope beam expander is used to amplify the entrance light to fill the back aperture in order to insure maximum NA. The field of view (FOV) of the collimator system is defined by the FOV of the micro-objective system multiplied by the transverse magnification of the micro-objective system, which is the same as the MEMS mirror effective reflecting area.

The probe sketch with MEMS device integrated is shown in Figure 7. In particular, the 2 V grooves cut on the round probe help to adjust the positioning tolerance of the MEMS device to the lens (X, Y and Z-decenter, Theta and Phi-tilt), thereby increasing the mechanical freedom dimension for the probe inner components.

Since the perfect overlap of pump and Stokes beams in the CARS microendoscope is a prerequisite for CARS signal generation, any slight misalignment in one of the eleven different glass components in the micro-objective system would lead to catastrophic chromatic aberration in the CARS microendoscope probe. Precise assembly and performance testing are needed to ensure each component is manufactured strictly to the specifications and integrated into the micro-objective system within the specified tolerance budget. We optimized the design by varying the curvature and air gap between glass components, and performed the Monte Carlo simulation of the CARS microendoscope probe system with the parameters distributed as follows: 0.1 mm for radius tolerance, 0.015 mm for sensitive surface radius tolerance, 0.025 mm for the airspace and glass thickness tolerance, 0.015 mm for thickness tolerance of the sensitive surface, 0.0005 for the index tolerance, 0.8% for V-number, 0.25 fringe for surface irregularity, 8 μm TIR for element wedge, 0.001 radians for element tilt, and 20 μm for element decenter. We have shown that the performance of the microendoscope probe is MTF 0.2 640lp/mm and RMS spot radius 1 µm@98%, [this is difficult to follow] indicating that the image resolution is guaranteed within 1 μm with our as-built probe. As-built image performance of CARS microendoscope probe is shown in Figure 8A and Figure 8B. The image performance of the collimator system is shown in Figure 9A and 9B. The spot diagram (which presents the resolution) is within 0.400 µm in +/- 0.5 degree field angle. MTF (modulation transfer function) is sharp and indicates that the chromatic aberration is well corrected and image performance is diffraction limited. The image performance of the micro-objective system is shown in Figure 10A and 10B. High spatial overlap on sample image side is achieved with resolution 0.46 µm at the center of the field of view and 0.57 µm at the marginal field of view.

We will complete Aim 1C and Aim 2 in year 2 and year 3.

C. Assemble and characterize the all-fiber-based CARS microendoscope system.

Aim 2: To evaluate the ability of our CARS microendoscope to image cavernous nerves and prostate surgical margins *in vivo* using intrinsic CH2-based molecular contrast.

A. Evaluate the utility of the CARS microendoscope to image patient surgical specimens ex vivo and transgenic mouse model for prostate cancer (TRAMP) mice in vivo.
B. Develop algorithms for image quantification and characterization of prostate and periprostatic tissues in TRAMP mice.
C. Validate the performance of microendoscope imaging and image analysis algorithms to identify prostate and periprostatic tissue in vivo.

Key Research Accomplishments

1. Examined collection efficiency and CARS imaging by single fiber to test the feasibility of fiber imaging
2. Improved collection efficiency and CARS imaging with customized fiber bundle composed of 6 multimode fibers and 18 multimode fibers
3. Design and manufacture of a miniaturized microendoscope probe as originally proposed
4. Monte Carlo simulation of the CARS microendoscope probe system to ensure the performance

Reportable Outcomes

1. Three conference presentations at SPIE Photonics West conference

    1) Zhengfan Liu, Zachary A. Satira, Xi Wang, Xiaoyun Xu, Xu Chen, Kelvin Wong, Shufen Chen, Jianguo Xin, Stephen T.C. Wong. Fiber bundle-based endomicroscopy prototype with two collection channels for simultaneous multimodal coherent anti-Stokes Raman scattering and second-harmonic generation imaging, Multiphoton Microscopy in the Biomedical Sciences XIV. Proceedings of the SPIE, Oral Presentation, 2014 (Accepted)
    2) Zhengfan Liu, Zhiyong Wang, Xi Wang, Xiaoyun Xu, Xu Chen, Jie Cheng, Xiaoyan Li, Shufen Chen, Jianguo Xin, Stephen T.C. Wong. Fiber bundle based probe with polarization for coherent anti-Stokes Raman scattering microendoscopy imaging, Multiphoton Microscopy in the Biomedical Sciences XIII. Proceedings of the SPIE, Oral Presentation, 85880F, 2013
    3) Xu Chen, Xi Wang, Xiaoyun Xu, Jie Cheng, Zhengfan Liu, Sheng Weng, Michael J. Thrall, Alvin C. Goh, Daniel T. McCormick, Kelvin Wong, Stephen T.C. Wong. Miniaturized CARS Microendoscope Probe Design for Label-free Intraoperative Imaging, Multiphoton Microscopy in the Biomedical Sciences XIII. Proceedings of the SPIE, Oral Presentation, 2014 (Accepted)

2. This DOD award supports three postdoctoral trainees (Xu Chen, Xiaoyun Xu, and Xi Wang)

3. This award also provides research opportunities for one masters student (Zachary Satira, graduated Oct 2013) and one PhD student (Zhengfan Liu)

Conclusion

Label-free CARS microendoscopy with µm resolution and miniaturized optics has the potential to revolutionize surgery with minimal invasiveness for *in situ* cancer diagnosis. Our group is pioneering the development of a miniaturized fiber-based CARS system to assist label-free differentiation of prostate tissues and CNs during robotic-assisted radical prostatectomy. To improve the efficiency of laser delivery and signal collection, we have tested the performance of fiber bundles in 6 and 18-multimode fiber configurations, versus single fiber. We have shown the feasibility of fiber-based systems for tissue imaging. We have completed the design of the miniaturized microendoscope probe and are currently in the phase of completing its fabrication. With the smallest transverse diameter (14mm) of a CARS microendoscope probe ever reported, and 1 µm resolution to promise clear cellular morphological imaging, this round-shaped CARS microendoscope probe has great potential to be applied in radical prostatectomy. The next step will be assembly and characterization of the all-fiber-based CARS microendoscope prorotype after we receive the microendoscope probe and fiber lasers. We

will then evaluate the utility of the microendoscope system by imaging patient surgical specimens as well as prostatic and periprostatic tissues in mouse and rat models.

References

1. Zhengfan Liu, Zachary A. Satira, Xi Wang, Xiaoyun Xu, Xu Chen, Kelvin Wong, Shufen Chen, Jianguo Xin, Stephen T.C. Wong. Fiber bundle-based endomicroscopy prototype with two collection channels for simultaneous multimodal coherent anti-Stokes Raman scattering and second-harmonic generation imaging, Multiphoton Microscopy in the Biomedical Sciences XIV. Proceedings of the SPIE, Oral Presentation, 2014 (Accepted)
2. Zhengfan Liu, Zhiyong Wang, Xi Wang, Xiaoyun Xu, Xu Chen, Jie Cheng, Xiaoyan Li, Shufen Chen, Jianguo Xin, Stephen T.C. Wong. Fiber bundle based probe with polarization for coherent anti-Stokes Raman scattering microendoscopy imaging, Multiphoton Microscopy in the Biomedical Sciences XIII. Proceedings of the SPIE, Oral Presentation, 85880F, 2013
3. Zhiyong Wang, Liang Gao, Pengfei Luo, Yaliang Yang, Ahmad A. Hammoudi, Kelvin K. Wong, and Stephen T. C. Wong. Coherent anti-Stokes Raman scattering microscopy imaging with suppression of four-wave mixing in optical fibers. Optics Express, 19(9), 7960-7970 (2011)
4. Xu Chen, Xi Wang, Xiaoyun Xu, Jie Cheng, Zhengfan Liu, Sheng Weng, Michael J. Thrall, Alvin C. Goh, Daniel T. McCormick, Kelvin Wong, Stephen T.C. Wong. Miniaturized CARS Microendoscope Probe Design for Label-free Intraoperative Imaging, Multiphoton Microscopy in the Biomedical Sciences XIII. Proceedings of the SPIE, Oral Presentation, 2014 (Accepted)
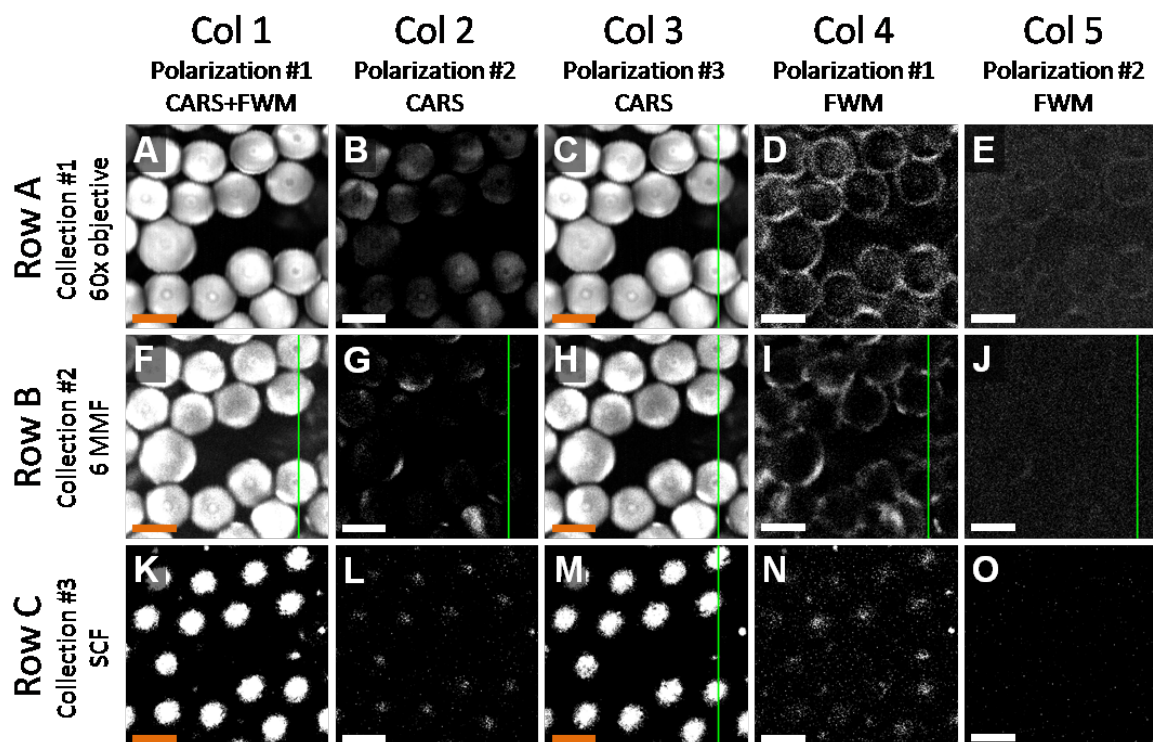
Appendices

Figure 1. CARS and FWM images of 10 μm PEBs. Row A, CARS microscope; Row B, six MMF collection based CARS microendoscope; Row C, single central fiber collection based CARS microendoscope; Col 1, CARS and FWM images in Polarization #1; Col 2, CARS images in Polarization #2; Col 3, CARS images in Polarization #3; Col 4, FWM images in Polarization #1; Col 5, FWM images in Polarization #2. Scale bar is 10 μm.
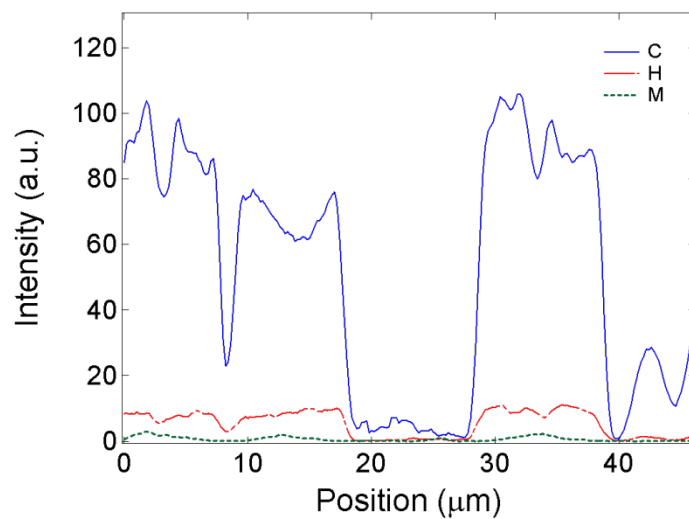
Figure 2. Intensity profiles normalized to same PMT gain value along vertical green lines in the images of Column 3 (CARS signals in Polarizaiton #3) of Figure 1.
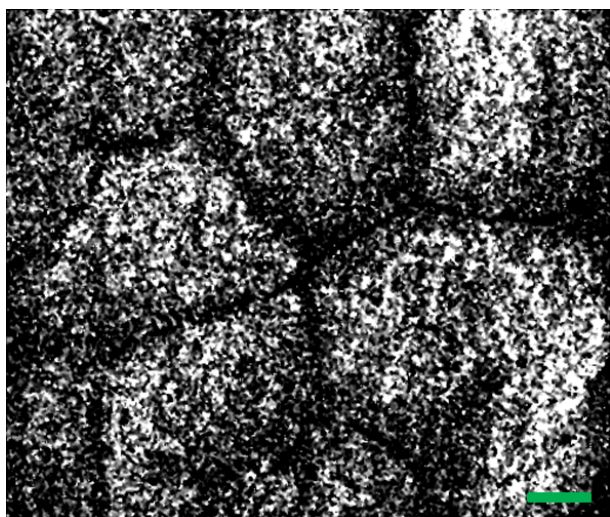

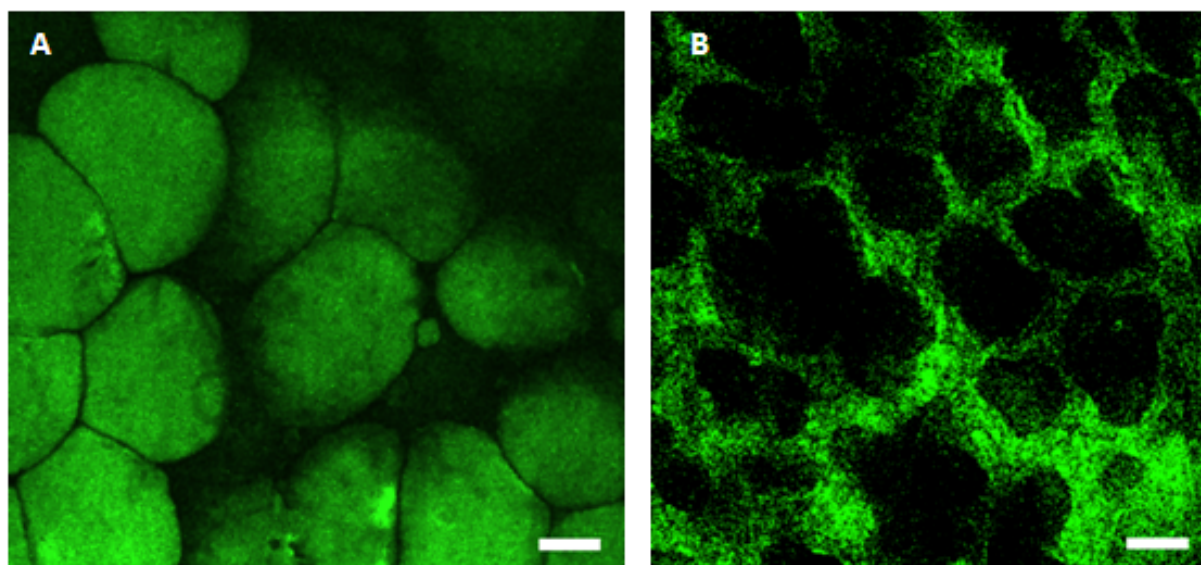Figure 3. CARS image of mouse adipocytes collected by single fiber. Scale bar: 20 um.

Figure 4. CARS images of tissue samples at the lipid band (2845 cm-1). (A) Mouse skin tissue, scale bar: 10 µm. (B) Mouse lung tissue, scale bar: 20 µm.
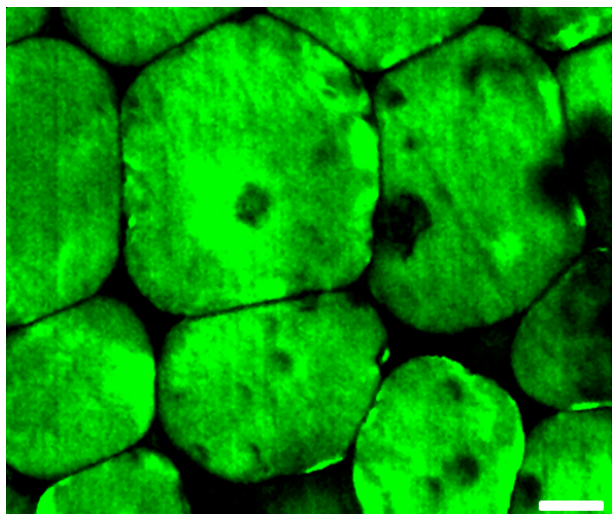

Figure 5. Mouse adipocyte collected by the 18 MMF. Scale bar is 20 µm.
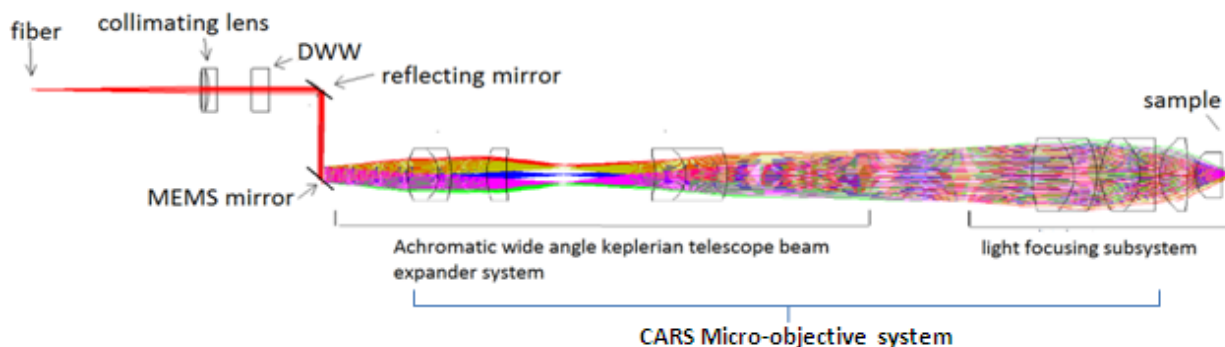

Figure 6: The optical imaging path schematic of CARS Microendoscope probe.
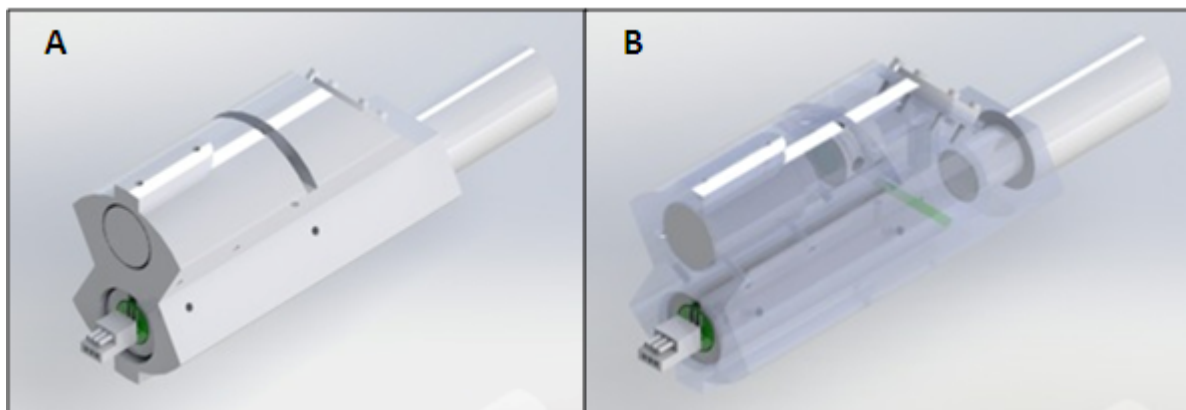

Figure 7: Probe sketch. (A) Mechanical system outline drawing of the CARS microendoscope probe; (B) Transparent view of the CARS microendoscope probe

Figure 8: As-built image performance of CARS microendoscope probe. (A) MTF performance; (B) spot diameter at different FOVs.

Figure 9: As-built image performance of the collimator system. (A) MTF performance; (B) spot diagram.

Figure 10: As-built image performance of the micro-objective system. (A) MTF performance; (B) spot diagram.